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EFFECTS OF MYCOTOXINS ON ANTIOXIDANT STATUS AND IMMUNITY

[1*]Peter F. Surai and [2]Julia. E. Dvorska
[1]Avian Science Research Centre, Scottish Agricultural College, Auchincruive, Ayr, Scotland, UK and [2]Sumy National Agrarian University, Sumy, Ukraine

Effects of mycotoxins on antioxidant systems

A delicate balance between antioxidants and pro-oxidants in the body in general and specifically in the cell is responsible for regulation of various metabolic pathways leading to maintenance of immunocompetence, growth and development and protection against stress conditions associated with commercial poultry production (Surai and Dvorska, 2001). This balance can be regulated by dietary antioxidants, including vitamin E (Surai *et al.,* 1999), carotenoids (Surai and Speake, 1998; Surai *et al.,* 2001) and selenium (Se) (Surai, 2000). On the other hand, nutritional stress factors have a negative impact on this antioxidant/pro-oxidant balance. In this respect mycotoxins are considered to be among the most important feed-borne stress factors.

It is not clear at present whether mycotoxins stimulate lipid peroxidation directly by enhancing free radical production or if the increased tissue susceptibility to lipid peroxidation is a result of a compromised antioxidant system. It seems likely that both processes are at work. In most cases lipid peroxidation in tissues caused by mycotoxins was associated with decreased concentrations of natural antioxidants. For example, in an experiment with quail, levels of the primary liver antioxidants (α–tocopherol, γ–tocopherol, carotenoids and ascorbic acid) were significantly decreased as a result of T-2 toxin consumption (Dvorska and Surai, 2001; Figure 1).

Similarly, the presence of T-2 toxin in the diet decreased the concentration of α–tocopherol in the chicken liver (Hoehler and Marquardt, 1996). T-2 toxin consistently depressed concentrations of vitamin E in chicken plasma (Coffin and Combs, 1981). Addition of micelle-promoting compounds (taurocholic, monoolein, and oleic acids) alleviated depression in plasma vitamin E, indicating interference of T-2 toxin with micelle formation during vitamin E absorption. Similarly, aflatoxin B_1 (AFB_1) in the feed interfered with the accumulation of carotenoids in chicken tissues (Schaeffer *et al.,* 1988) inducing pale bird syndrome in birds. In fact, AFB_1 caused a significant depression of lutein in the toe web, liver, serum and mucosa (Schaeffer *et al.,* 1988a). Pigment restoration was accomplished by feeding the same diet supplemented with lutein (70 mg/kg). In young chickens AFB_1 reduced the lutein content of jejunal mucosa up to 35% while serum lutein was reduced up to 70% (Tyczkowski and Hamilton, 1987), suggesting that AFB_1 interfered with the absorbtion, transport and deposition of carotenoids. More precisely, AFB_1 impaired lutein absorption in chickens (Tyczkowski and Hamilton, 1987a). In similar fashion, ochratoxin A (OTA) was shown to affect

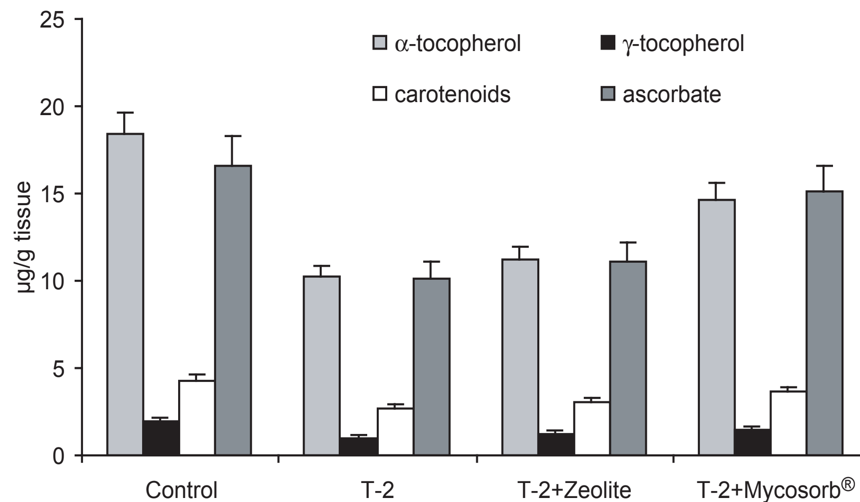

Figure 1. Effect of T-2 toxin with and without supplemental toxin adsorbents on antioxidant concentrations in quail liver (adapted from Dvorska and Surai, 2001).

carotenoid assimilation in chickens. Again, depression in uptake of carotenoids by intestinal mucosa and depressed transport in serum were considered to be important mechanisms of AFB_1 action on carotenoid metabolism (Schaeffer *et al.,* 1988; Huff and Hamilton, 1975).

In general, **malabsorption syndrome** is considered a common result of mycotoxicoses. For example, aflatoxicosis, ochratoxicosis and T-2 toxicosis were produced by feeding diets containing graded concentrations of the appropriate toxin to broiler chickens from hatching until 3 weeks of age (Osborne *et al.,* 1982). In this experiment AFB_1, even at levels lower than needed for growth inhibition, produced a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia, and decreased concentrations of bile salts and pancreatic enzymes. T-2 toxin also produced malabsorption, but at concentrations higher than required to inhibit growth. Ochratoxicosis produced mainly hypocarotenoidemia (Osborne *et al.,* 1982). It is postulated that the decreased level of vitamin A in the quail liver as a result of T-2 toxin consumption (Dvorska and Surai, 2001) is also a reflection of the decreased intestinal absorption of fat soluble nutrients.

The presence of OTA in the diet significantly decreased the concentration of α–tocopherol in the chicken liver (Hoehler and Marquardt, 1996). Furthermore, aflatoxin-treated barrows had decreased serum tocopherol and retinol concentrations compared with control and pre-test values, and decreased tocopherol concentration in cardiac tissue (Harvey *et al.,* 1994). Aurofusarin decreased vitamin E and carotenoid concentration in quail egg yolk (Dvorska *et al.*, 2001a), in the liver, yolk sac membrane and other tissues of newly hatched quail (Dvorska *et al.,* 2002; Dvorska *et al.,* 2003; Dvorska and Surai, 2004). It is interesting to note that the carotenoid and vitamin A concentrations in the quail liver were also decreased when the maternal diet was supplemented with aurofusarin.

A pro-oxidant effect of mycotoxins in many cases could be mediated through changes in reduced glutathione (GSH) concentration. For example, Rizzo *et al.* (1994) demonstrated that T-2 toxin decreased GSH in rat liver. Treatment of fasted mice with a single dose of T-2 toxin (1.8 or 2.8

mg/kg BW) by oral gavage led to a marked decrease in hepatic GSH (Atroshi *et al.,* 1997). In male broiler chicks, hepatic GSH concentration decreased after 7 days of treatment (1.5 mg T-2 toxin/kg BW/day) (Leal *et al.,* 1999). Acute exposure of mice to T-2 toxin (4 mg/kg, s.c.) resulted in a progressive decrease in hepatic GSH, reaching a minimum 6-8 hrs after toxin administration (Fricke and Jorge, 1991). Intraperitoneal administration of AFB_1 to rats (2 mg/kg) was also associated with decreased GSH in the liver. In contrast, in 3-week-old male chickens daily aflatoxin gavage (2 mg/kg BW, in corn oil) for 5 and 10 days elevated hepatic GSH; and renal GSH was elevated after 10 days (Beers *et al.,* 1992a). Similarly, hepatic GSH increased 2 and 8 hrs following a single AFB_1 dose and continued to increase through five daily doses of AFB_1 (Beers *et al.,* 1992b).

There was GSH depletion in cultured rat hepatocytes as a result of AFB_1 toxicosis (Liu *et al.,* 1999). Similarly, consumption of OTA for two weeks was associated with a depletion of GSH from the mouse liver (Atroshi *et al.,* 2000). The mycotoxin patulin also decreased GSH concentration in rat hepatocytes (Busbee *et al.,* 1999). Considering that glutathione is responsible for the maintenance of redox status of the cell (Sies, 1999) and therefore participates in regulation of gene expression (Arrigo, 1999), changes in GSH status could be detrimental.

One of the most important mycotoxin actions is their effect on antioxidant enzymes. Depending on experimental conditions (species, doses, route and duration of administration, concentrations of other antioxidants etc.), antioxidant enzyme activities can be increased in response to oxidative stress or decreased by direct or indirect action of mycotoxins. For example, treatment of pig kidney cells with 50 mM fumonisin B_1 (FB_1) for 24 hrs significantly decreased cellular GSH and increased the activities of glutathione reductase (Kang and Alexander, 1996). The activities of glutathione peroxidase (GSH-Px), catalase, and Cu/Zn-superoxide dismutase (SOD) were not changed by this treatment. Oral administration of T-2 mycotoxin to rats (1.25 mg/kg) for 5 days decreased the activity of liver glutathione-S-transferase (Ahmed and Ram, 1986). In contrast, feeding a single dose of T-2 toxin (2 mg/kg BW) to rats increased activities of GSH-shuttle enzymes including GSH-Px, glutathione reductase and glucose-6-phosphate dehydrogenase (Suneja *et al.,* 1989), probably reflecting an adaptive response to oxidative stress. On the other hand, when male rats were given a diet deficient in vitamins C and E and Se and were administered orally a single dose of deoxynivalenol (DON) or T-2 toxin, there was a significant decrease in activities of GSH-Px, catalase, SOD and glutathione reductase (Rizzo *et al.,* 1994). Activity of GSH-Px in rat blood was decreased due to consumption of AFB_1 (Choi *et al.,* 1995). Administration of AFB_1 to rats (2 mg/kg intraperitoneally) caused a significant decrease in the activities of SOD, catalase, GSH-Px, glutathione-S-transferase and glutathione reductase in liver (Rastogi *et al.,* 2001). A significant increase in SOD activity occurred in the liver following AFB_1 exposure of the ducks (Barraud *et al.,* 2001).

When considering detrimental effects of mycotoxins on antioxidant systems it is necessary to be aware that combinations of several mycotoxins can be more toxic than individual mycotoxins, as was shown for a combination of T-2 toxin and OTA (Kubena *et al.,* 1989).

One of the most important targets for mycotoxins is embryonic development. Since chicken embryo tissues contain high levels of PUFA, they are vulnerable to peroxidation and oxidative stress caused by mycotoxins could be lethal. As mentioned previously, aurofusarin increased late mortality of quail embryos (Dvorska *et al.,* 2001a). Furthermore, contamination of the diet with T-2 toxin markedly decreased egg production and impaired hatchability (Tobias *et al.,* 1992). Confirmation of a possible association of this effect with oxidative stress came from data

indicating that increased dietary vitamin E during the first week of the experiment significantly decreased the number of infertile eggs and significantly improved the hatching percentage (Tobias *et al.,* 1992).

Increased lipid peroxidation as a consequence of mycotoxicoses

As illustrated in Table 1, OTA has a stimulating effect on lipid peroxidation. In most of cases, accumulation of thiobarbituric acid reactive substances (TBARS) was used as a measurement of lipid peroxidation. Ethane exhalation, EPR-registered free radicals, hydroxyl radical formation, single-strand cleavage DNA, DNA adduct formation as well as LDH release were also used to confirm pro-oxidant properties of OTA. Various *in vitro* and *in vivo* systems were also used including liver microsomes, phospholipid vesicles, primary cell cultures, whole organ and whole body measurements.

T-2 toxin was also shown to have pro-oxidant properties (Table 2). Those properties were confirmed with rat, mouse and quail liver tissue and yeast. TBARS accumulation was the method used in most of the studies, however, conjugate diene formation and DNA fragmentation were also demonstrated. Effect of AFB_1 on lipid peroxidation has been studied in rat liver and kidney as well as in cultured hepatocytes and in an *in vitro* model

Table 1. Stimulation of lipid peroxidation by ochratoxin A.

Mycotoxin	*Tissue*	*Lipid peroxidation measurement*	*Protective effect of antioxidants*	*References*
OTA *in vitro*	Rat liver microsomes	TBARS ↑	-	Rahimtula *et al.,* 1988, Gautier *et al.,* 2001; Khan *et al.,* 1989
OTA in feed	Rats	Ethane exhalation ↑	-	Rahmitula *et al.,* 1988
OTA in feed	Rats	MDA in serum, liver, kidney ↑	-	Meki and Hussein, 2001
OTA *in vitro*	Phospholipid vesicles	TBARS ↑, oxygen uptake ↑	-	Omar *et al.*, 1990
OTA and its analogs	*Bacillus brevis* bacteria	Free radical generation (EPR) ↑	Vitamin E	Hoehler *et al.,* 1996
OTA in feed	Chicken liver	TBARS ↑	-	Hoehler and Marquardt, 1996; Hoehler *et al.*, 1997
OTA *in vitro*	Vero cells in culture	TBARS ↑	SOD, catalase aspartame	Baudrimont *et al.,* 1997a; 1997b
OTA in diet	Rat liver	TBARS ↑	-	Hoehler *et al.,* 1997
OTA *in vitro*	A model oxidation system	OH• ↑, single-strand cleavage DNA ↑	-	Gillman *et al.,* 1999
OTA *in vitro*	Astrocytes and neurons	TBARS ↑, LDH release ↑	-	Belmadani *et al.,* 1999
OTA in feed	Mouse and rat kidney	DNA adduct formation ↑	Retinol, ascorbic acid, vitamin E	Grosse *et al.,* 1997
OTA in feed	Rats	DNA adduct formation ↑	Aspartame	Creppy *et al.,* 1998
OTA *in vitro*	Rat kidney microsomes	TBARS ↑	-	Gautier *et al.*, 2001

Table 2. Stimulation of lipid peroxidation by T-2 toxin.

Mycotoxin	*Tissue*	*Lipid peroxidation measurement*	*Protective effect of antioxidants*	*References*
T-2 in feed	Rat liver	TBARS ↑	Se, ascorbic acid, vitamin E	Tsuchida *et al.,* 1984; Suneja *et al.,* 1989; Schuster *et al.,* 1987; Rizzo *et al.,* 1994
T-2 in feed	Rat liver nuclei	TBARS ↑	-	Ahmed and Ram, 1986
T-2 in feed	Mouse liver	TBARS ↑	-	Karppanen *et al.,* 1989
T-2 in feed	Mouse	MDA in liver after 24-48 hrs after supplementation↑		Vila *et al.,* 2002
T-2 in feed	Mouse liver	DNA fragmentation ↑	CoQ_{10} and vitamin E	Atroshi *et al.,* 1997
T-2 in feed	Quail liver	TBARS ↑	-	Dvorska and Surai, 2001
T-2 in feed	Rat tissues	Conjugate diene ↑	-	Chang and Mar, 1988
T-2 in feed	Chicken	Liver MDA ↑	Lycopene	Leal *et al.,* 1999
T-2 in feed	Chicken, duck, goose	Liver MDA ↑		Mezes *et al.,* 1999

system (Table 3). Similar to the examples above, TBARS accumulation was substantially increased as well as conjugate diene production. At the same time GSH concentration and activities of antioxidant enzymes significantly declined as a result of AFB_1 action.

Fumonisin B_1 also stimulated lipid peroxidation in rat liver, rat liver nuclei fraction, primary rat hepatocytes, Vero cells in culture and phosphatidyl choline (PC) bilayers. In those systems TBARS accumulation and DNA strand breaks were increased (Table 4). DON increased TBARS formation in rat and mouse liver and decreased GSH in rat brain and spleen. There are also data indicating pro-oxidant properties of zearalenone (Karagezyan *et al.,* 1995; Ghedira-Chekir *et al.,* 1999) and citrinin (Ribeiro *et al.,* 1997). Aurofusarin has been shown to decrease antioxidant defences and stimulate lipid peroxidation in quail egg and tissues of newly hatched quail (Dvorska *et al.,* 2002; 2003; Dvorska and Surai, 2004).

It is clear from these data that mycotoxins strongly promote lipid peroxidation in various *in vitro* and *in vivo* systems. This effect was obvious no matter which measurement was used to assess the process of lipid peroxidation.

Mycotoxins and apoptosis

The maintenance of tissue homeostasis involves the removal of superfluous and damaged cells. This process is often referred to as **programmed cell death** or **apoptosis***,* since it is thought that cells activate an intrinsic death program contributing to their own demise (Sastre *et al.,* 1996). Several processes, such as initiation of death signals at the plasma membrane, expression of pro-apoptotic oncoproteins, activation of death proteases, endonucleases etc., ultimately coalesce to a common irreversible execution phase leading to cell demise. A balance between cell death and cell survival factors plays a major role in the decision process as to whether a cell should live or die (Ray *et al.,* 2000).

Apoptosis is distinguishable from necrosis. When cell death is induced by osmotic, physical or chemical damage, early disruption of external and internal membranes takes place with subsequent liberation of denatured

Table 3. Stimulation of lipid peroxidation by aflatoxin.

Mycotoxin	*Tissue*	*Lipid peroxidation measurement*	*Protective effect of antioxidants*	*References*
In feed	Rat liver	TBARS ↑, conjugated dienes ↑	Se , vitamin E	Shen *et al.,* 1994
In feed	Rat liver	MDA ↑, NO↑	melatonin - loaded chitosan	El-Gibaly *et al.,* 2003
In feed	Rat liver	MDA ↑, NO ↑	melatonin	Meki *et al.,* 2001
In feed	Rat testis	MDA ↑	Vitamin E	Verma and Nair, 2001
In vitro	Cultured rat hepatocytes	TBARS ↑, LDH release ↑	SOD, catalase	Shen *et al.,* 1995
In feed	Rat liver	TBARS ↑	*Semecarpus anacardium* nut extract	Premalatha *et al.,* 1997
In vitro	Cultured primary rat hepatocytes	TBARS ↑ ROS formation ↑	*Silvia miltorrhiza* extract	Liu *et al.,* 1999
Intraperitoneally	Rat liver	TBARS ↑	Vitamin E, ternatin	Souza *et al.,* 1999
In feed	Rat liver	GSH-Px ↓	Se, vitamin E	Choi *et al.,* 1995
Intraperitoneally	Rat liver and kidney	TBARS ↑	Picroliv	Rastogi *et al.,* 2001
Intraperitoneally	Rat liver	GSH, SOD, Catalase, GSH-Px ↓	-	Rastogi *et al.,* 2001b
In vitro	Primary rat hepatocytes	TBARS ↑, GSH ↓, ROS generation ↑	-	Yang *et al.,* 2000

proteins into the cellular space and induction of an inflammatory response in the vicinity of the dying cell (Sastre *et al.,* 1996). In contrast, apoptosis is characterised by cell shrinkage, nuclear pyknosis, chromatin condensation, DNA cleavage into fragments of regular sizes and activation of proteases called caspases (Dare *et al.,* 2001).

Reactive oxygen species (ROS) are thought to play a major role in apoptosis (Hockenbery *et al.,* 1993; Ratan *et al.,* 1994), being involved in both initiation and execution of apoptosis (Herdener *et al.,* 2000). GSH depletion increases the percentage of apoptotic cells in a given population; and increased GSH concentration is shown to decrease the percentage of apoptosis in fibroblasts (Sastre *et al.,* 1996). In fact, GSH depletion sensitises cells for intracellular induction of apoptosis (Zucker and Bauer, 1997). Therefore, a decrease in GSH, or an increase in GSSG or perhaps a change in the ratio of the two constitutes a trigger for apoptosis (Beaver and Waring, 1995). For example ROS from mitochondria can cause apoptosis after GSH depletion (Zucker *et al.,* 1997). Therefore, apoptosis is induced by oxidative damage either directly from oxygen free radicals or hydrogen peroxide or from their generation in cells by injurious agents. For example, hydrogen peroxide is considered a common mediator for the apoptosis induced by various anticancer drugs (Simizu *et al.,* 1998). In line with those findings there are data showing protective effects of catalase and SOD from different inducers of apoptosis (Sandstrom and Buttke, 1993; Herdener *et al.,* 2000). Indeed, intracellular induction of apoptosis depends on ROS production and can be efficiently blocked by antioxidants (Langer *et al.,* 1996; Schaefer *et al.,* 1995).

As previously mentioned, in many cases mycotoxins decreased cellular GSH, which

Table 4. Stimulation of lipid peroxidation by fumonisin, deoxynivalenol, aurofusarin and citrinin.

Mycotoxin	*Tissue*	*Lipid peroxidation measurement*	*Protective effect of antioxidants*	*References*
Fumonisin	Rat liver nuclei	TBARS ↑, DNA strand breaks ↑	Catalase, mannitol	Sahu *et al.,* 1998
Fumonisin B_1	Primary rat hepatocytes	TBARS ↑	Vitamin E	Abel and Gelderblom, 1998
Fumonisin B_1	Rat liver	TBARS ↑	-	Abel and Gelderblom, 1998; Lemmer *et al.,* 1999
Fumonisin B_1	Phosphati-dyl choline bilayers	Rate of peroxidation ↑, free radical Formation ↑, Acceleration of chain formation ↑	-	Yin *et al.,* 1998
Fumonisin B_1	Vero cells	TBARS ↑	-	Abado-Becognee *et al.,* 1998
Fumonisin B_1	Glioma cells, Mouse fibroblasts	TBARS ↑, DNA fragmentation	-	Mobio *et al.,* 2003
Fumonisin B_1	Macrophage cell line	MDA↑	-	Ferrante *et al.,* 2002
DON in feed	Rat liver	TBARS ↑	Se, ascorbic acid, vitamin E	Rizzo *et al.,* 1994
DON + 3-AcDON in feed	Mouse liver	TBARS ↑	-	Karppanen *et al.,* 1989
Aurofusarin in feed	Quail egg yolk	TBARS ↑	-	Dvorska *et al.,* 2001b, Dvorska, 2001
Aurofusarin in feed	Liver of newly hatched quail	TBARS ↑	-	Dvorska *et al.,* 2002 Dvorska and Surai, 2004
Zearalenone injected IV	Rat liver	TBARS ↑	-	Karagezyan *et al.,* 1995
Zearalenone *in vitro*	Vero cells	TBARS ↑	Vitamin E	Ghedira-Chekir *et al.,* 1999
Citrinin in feed	Rat liver	TBARS ↑	-	Ribeiro *et al.,* 1997

can trigger apoptosis. In general, T-2 toxin is the most potent apoptotic agent among mycotoxins. However, there are also reports indicating apoptosis caused by FB_1, OTA and AFB_1. For example, based on the DNA fragmentation profile in gel electrophoresis and the morphological changes seen in electron microscopy, the induction of apoptotic nuclear changes by various mycotoxins was investigated in HL-60 human promyelotic leukemia cells (Ueno *et al.,* 1995). The results showed that T-2 toxin, nivalenol (NIV), DON, OTA, citrinin, AFB_1 and some other mycotoxins induced DNA fragmentation. Morphological evidence of apoptosis was related to the toxicity of the mycotoxins and more toxic NIV and DON resulted in more late stage apoptotic events than FB_1. The results suggested that DNA damage and apoptosis rather than plasma membrane damage and necrosis may be responsible for the observed cytotoxicity.

Fumonisin B_1, T-2 toxin, fusarenon-X and DON could induce apoptosis of liver cells, kidney cells, gastrointestinal epithelial cells and immunological cells as well as several cell lines. The possible mechanisms of apoptosis induction are not well understood. It seems likely that sphingol, p21 gene, protein kinase and intracellular Ca^{2+} level might be involved in the cellular apoptosis induction by *Fusarium* mycotoxins (Wang and Zhang, 2000).

T-2 TOXIN

The rank order of the potency of trichothecene mycotoxins to induce internucleosomal DNA fragmentation was found to be T-2, satratoxin G, roridin A >> diacetoxyscirpenol > baccharin B-5 >> nivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, fusarenon-X, baccharin B-4 vehicle control (Nagase *et al.,* 2001). The authors showed that T-2-induced apoptosis involved activation of caspase-3 through cytosolic accumulation of cytochrome c along with caspase-9 activation. Both T-2 and HT-2 induced apoptosis after 24 hrs in HL-60 cells, with T-2 being somewhat more potent than HT-2 and the apoptotic process was almost completely blocked in the presence of a caspase inhibitor (Holme *et al.,* 2003). Development of apoptosis and changes in lymphocyte subsets were examined in thymus, mesenteric lymph nodes and Peyer's patches of mice up to 24 hrs after oral inoculation with T-2 toxin (10 mg/kg). The degree of lymphocyte apoptosis was prominent in the thymus, moderate in the Peyer's patches, and somewhat mild in the mesenteric lymph nodes (Nagata *et al.,* 2001). Cytocentrifugation and light microscopy of leukocyte-enriched cell samples from the pronephros (the primary hematopoietic compartment in the fish) demonstrated T-2-related increases in apoptotic bodies and the apoptotic probes confirmed apoptosis in fish (Gogal *et al.,* 2000). Female mice were treated orally with T-2 toxin (10 mg/kg BW) and thymus and spleen were examined to detect apoptotic changes (Shinozuka *et al.,* 1997). The number of apoptotic lymphocytes in the thymus increased dramatically from 9 to 24 hrs after treatment and began to increase in the spleen at 12 hrs after treatment indicating that thymus was more sensitive to apoptosis than spleen. In another experiment, mice were given 5 mg/kg BW of T-2 toxin and were killed 12 hrs later (Ihara *et al.,* 1997). Massive cellular destruction was observed in the thymus and spleen and electron microscopy revealed the presence of apoptotic bodies. Similarly, in the liver of mice given 2.5 mg/kg T-2 toxin and killed 2 hrs later, the induction of apoptotic cellular lesions was observed. Electron microscopic characteristics of damaged lymphocytes were shrinkage of the cell body, nuclear chromatin condensation and fragmentation (Li *et al.,* 1997). Of nine trichothecene mycotoxins tested, T-2 toxin was the most potent agent to induce apoptosis in the thymus in young female mice (Islam *et al.,* 1998; 1998a). It is interesting that both the acetyl group at the C-4 position and the isovaleryl or 3'-hydroxy isovaleryl group at the C-8 position of the T-2 toxin molecule are important for inducing apoptotic changes in the thymus (Islam *et al.,* 1998). T-2 toxin was shown to induce apoptosis in the hematopoietic (bone marrow) tissues of female mice (Shinozuka *et al.,* 1998). It should be noted that the gastrointestinal tract is also sensitive to trichothecene-induced apoptosis, since such changes were observed in the gastric mucosa, gastric glandular epithelium and intestinal crypt cell epithelium (for review see Bondy and Pestka, 2000). These changes could impair nutrient assimilation as well as decrease defences against enteric pathogens and endotoxin.

To examine the effect of T-2 toxin on the developing embryo, pregnant mice were dosed orally with T-2 toxin (3 mg/kg BW) at 11 days of gestation (Ishigami *et al.,* 1999). Ultrastructural changes characteristic of

apoptosis were observed. For example, in some layers of the central nervous system moderate pyknosis or karyorrhexis was seen. Using DNA fragmentation and fluorescence microscopy assays Yang *et al.* (2000a) demonstrated that rank order of trichothecene-mediated apoptosis is similar to their cytotoxicity. Therefore, cytotoxicity of trichothecene mycotoxins is mediated through an apoptotic process. In particular, the embryotoxic effect of T-2 toxin on the mouse fetus is mediated through apoptosis. T-2 toxin (2 mg/kg BW) was given orally to pregnant mice at various gestational days and the fetuses were examined 24 hrs later. T-2 toxin-induced apoptosis was detected. Since T-2 toxin readily crosses the placenta, T-2 toxin could directly cause fetal apoptosis (Ishigami *et al.,* 2001).

It was shown that in human promyelotic cell line HL-60, T-2 toxin induced apoptosis at 10 ng/ml levels within 2-6 hrs (Ueno *et al.,* 1995). Apoptosis in HL-60 cells induced by T-2 toxin was dose dependent when cells were treated with concentrations of 5-100 ng/ml for more than 2 hrs (Yoshino *et al.,* 1996). The authors suggested that the Ca^{2+} signal triggered by T-2 toxin is transduced by the activation of endonuclease and protease, and ultimately evokes apoptosis.

Molecular mechanisms of T-2 stimulation of apoptosis are not clear at present. However, recently it has been shown that trichothecene mycotoxins trigger a ribotoxic stress response that activates the stress-activated kinases c-Jun N-terminal kinase and/or p38 mitogen-activated kinase and induces apoptosis (Shifrin and Anderson, 1999). Such activation may signal cell survival or induce apoptosis in various cell types depending on the conditions, e.g. length of signal. However, the pathway leading from the activation of these kinases to the activation of caspases and apoptosis has not been elucidated and needs further research. Albarenque *et al.* (2001) suggested that the elevation of TNF-α mRNA expression may play an important role in T-2 toxin-induced epidermal cell apoptosis. The induction of c-fos and perhaps of c-jun mRNAs may be associated with T-2 toxin-induced epidermal cell apoptosis (Albarenque *et al.,* 2001).

FUMONISIN B_1

The effects of FB_1 on apoptosis in various *in vitro* and *in vivo* studies have received substantial attention over the last few years. For example, FB_1-treated keratinocytes developed morphological features consistent with apoptosis. In particular, they released nucleosomal DNA fragments into the medium 2-3 days after exposure to 0.1 mM FB_1 and showed increased DNA strand breaks (Tolleson *et al.,* 1996). FB_1 induced a significant and dose-related increase of modifications of DNA bases (8-OH-dG) and DNA fragmentation in both C6 glioma and mouse embryonic fibroblasts cells (Mobio *et al.,* 2003). In the same study, apoptotic C6 glioma cells were also observed after FB_1 incubation. Recently genes that inhibit FB_1-induced apoptosis in African green monkey kidney fibronlasts (CV-1 cells) and two mouse embryo fibroblasts have been identified (Jones *et al.,* 2001). Primary mouse embryo fibroblasts underwent apoptosis following FB_1 treatment (Ciacci-Zanella *et al.,* 1999). The authors also demonstrated that the tumour necrosis factor (TNF) pathway and caspases play an important role in FB_1-induced apoptosis. Effects of exposure of human fibroblasts to FB_1 were investigated. After 72 hrs of treatment, FB_1 (50 and 100 mM) induced DNA damage, an enhancement of caspase-3-activity and cleavage of poly(ADP-ribose)polymerase compared to controls (Galvano *et al.,* 2002).

The cytotoxicity and genotoxicity of FB_1 in rabbit kidney RK13 cells were examined. Exposure to FB_1 caused a significant increase in micronucleus frequency in a concentration- and in a time-dependent manner and an increased number of the cells were dying by the process of apoptosis (Rumora *et al.,* 2002). The author suggested that nanomolar

concentrations of FB_1 induced apoptosis, which subsequently may proceed to secondary necrosis. Treatment of monkey kidney cells (CV-1 cells) with FB_1 led to cell cycle arrest and apoptosis (Zhang *et al.,* 2001). It was suggested that the ability of FB_1 to alter gene expression and signal transduction pathways may be necessary for its carcinogenic and toxic effects.

In general, FB_1 induces apoptosis of hepatocytes and of proximal tubule epithelial cells. More advanced lesions in both organs were characterised by simultaneous cell loss (apoptosis and necrosis) and proliferation (Voss *et al.,* 2001). A dose-dependent increase in TNF-α–induced apoptosis was observed in porcine renal epithelial cells pretreated with FB_1. In particular, cells treated with FB_1 showed increased DNA fragmentation and terminal uridine nucleotide end labeling in response to TNF-α treatment (Johnson *et al.,* 2003). In the same study, FB_1 also increased DNA synthesis and resulted in cell cycle arrest in the G2/M phase of the cell cycle.

FB_1 toxicity caused induction of cytokine networks in liver with involvement of the TNF-α signaling pathway (Bhandari and Sharma, 2002). The authors suggested that increased expression of caspase 8 involved in the TNF-α signaling pathway may contribute to the apoptosis, whereas IL-1Ra induction could contribute to the proliferating effects observed in FB_1 toxicity. Similarly, FB_1 caused an activation of the cytokine network in liver, particularly the TNF-α signaling pathway, suggesting its involvement in hepatotoxic mechanisms (Bhandari *et al.,* 2002). Induction of apoptosis was suggested to be a consequence of ceramide synthase inhibition and disruption of sphingolipid metabolism by FB_1 (Dragan *et al.,* 2001). It seems likely that the elevated endogenous sphinganine acts as a contributing factor to the fumonisin-induced cell death (Yu *et al.,* 2001). Moreover recent results of Mobio *et al.* (2000) showed that cytotoxic concentrations of FB_1 induce cellular cycle arrest in phase G(2)/M in rat C6 glioma cells. Furthermore, under experimental conditions (9 or 18 mM FB_1 in the medium) there was an induced DNA fragmentation and laddering and many apoptotic bodies in glioma cells.

As mentioned above, FB_1 caused morphological changes (i.e., cell shrinkage, membrane blebbing) and time-dependent increases in DNA fragmentation characteristic for apoptosis. For example, Kim *et al.* (2001) demonstrated that FB_1 kills LLC-PK(1) kidney cells by inducing apoptosis. This included initial disruption of sphingolipid metabolism and accumulation of sphinganine (or a metabolite), which, in turn, induced expression of calmodulin. FB_1, but not AFB_1, induced the apoptosis of swine alveolar macrophages (AM) with evidence of DNA laddering and nuclear fragmentation. However, both FB_1 and AFB_1 exposure induced the expression of apoptosis-related heat shock protein 72 in AM (Liu *et al.,* 2002).

FB_1 has been shown to cause apoptosis in a variety of cell types and tissues, but the apoptotic potential of other fumonisins and fumonisin metabolites could substantially vary. For example, Seefelder *et al.* (2003) exposed human proximal tubule-derived cells (IHKE cells) to FB_1, FB_2, FB_3, hydrolyzed FB_1 and N-palmitoyl-hydrolyzed FB_1 and investigated caspase-3 activation, chromatin condensation and DNA fragmentation. The results demonstrated that all compounds led to increased sphinganine levels in IHKE cells, but only FB_1 was able to induce apoptosis. In the short-term studies, increases in tissue concentration of the ceramide synthase substrate sphinganine and the sphinganine:sphingosine ratio were correlated with apoptosis (Voss *et al.,* 2002).

Although it has been demonstrated that FB_1 induces apoptosis in many cell lines, the precise mechanism of apoptosis is not fully understood. For example, FB_1-induced apoptosis involves the activation of caspase 3, which is associated with the repression of protein kinase C and possibly its downstream

effectors, NF-κB and TNF-α (Gopee *et al.,* 2003).

After intravenous injection of male rats with FB_1 (1.25 mg/kg), hepatic and renal GSH concentrations were depressed and proliferation and apoptosis were observed in the outer medulla of the kidney cell (Lim *et al.,* 1996). When male mice were injected with FB_1 subcutaneously at doses of 0, 0.25, 0.75, 2.25 and 6.75 mg/kg BW daily for 5 days, a dose-dependent increase in apoptosis in liver and kidney was observed (Tsunoda *et al.,* 1998). Similar observations were described by Sharma *et al*. (1997). Male mice were injected subcutaneously with vehicle or 2.25 mg/kg/day of FB_1 for 5 days and sampled 1 day after the last treatment. FB_1 increased apoptotic cells in liver (Bhandari *et al.,* 2002). Similarly, histopathology revealed the occurrence of apoptosis in the liver of rats exposed to FB_1 (Pozzi *et al.,* 2001).

Multiple daily doses of FB_1 after surgery elevated the number of apoptotic hepatocytes in rats (Li *et al.,* 2000). Including FB_1 in the diets of rats increased hepatocellular and renal tubule epithelial cell apoptosis (Howard *et al.,* 2001). It is interesting that female rats demonstrated more sensitivity than male rats in the induction of hepatocellular apoptosis and mitosis. Mild toxic effects, including apoptosis, proliferation of bile duct epithelial cells, and early signs of fibrosis were noticed in the liver of rats fed FB_1 (25 mg/FB_1/kg diet; Gelderblom *et al.,* 2001). Six hours after administration of FB_1, marked morphologic changes of rat hepatocytes included the appearance of small vacuoles along the margin of the cell membrane. Electron microscopic analysis revealed margination of nuclear chromatin and swollen mitochondria with amorphous matrical deposit (Moon *et al.,* 2000). The authors suggested that FB_1-induced alteration of hepatocyte membrane lipid composition is an early key event in the FB_1 apoptotic effect. Therefore, the induction of tubular epithelial cell apoptosis was the primary response of the kidneys to dietary FB_1. Apoptosis was present at all doses (99-484 ppm for 28 days) in the kidney of male rats and occurred in females at doses of 153-484 ppm FB_1 (Tolleson *et al.,* 1996). Wistar rats were fed diets containing 0 (control) or 100 ppm FB_1 for 12 weeks. Necrosis and apoptosis of tubular epithelial cells in the kidney were observed and increased mitotic figures and lymphocytic infiltrate in the small intestine were found (Theumer *et al.,* 2002). Increased hepatocellular apoptosis was also detected in the mice consuming diets containing 72 and 143 mmol/kg FM_1 (Howard *et al.,* 2002).

FB_1 can induce renal injury and organ sphingolipid alterations in cattle. For example, renal lesions in FB_1-treated calves consisted of vacuolar change, apoptosis, caryomegaly, and proliferation of proximal renal tubular cells, as well as dilation of proximal renal tubules, which contained cellular debris and protein (Mathur *et al.,* 2001). Probably there is species-specificity in susceptibility to FB_1. For example, FB_1 was investigated in four groups of growing ducks, each receiving 0, 5, 15 or 45 mg/kg FB_1 by daily oral administration over 12 days. No sign of apoptosis was present in the liver or in peripheral blood lymphocytes and only moderate oxidative damage was noted (Bailly *et al.,* 2001).

The apoptosis-stimulating effect of FB_1 has been clearly demonstrated; however molecular mechanisms of this stimulation are not clear at present. Nevertheless, stimulation of lipid peroxidation by FB_1 and decreased antioxidant concentrations including GSH in tissues could lead to changes in redox status of the cell and trigger a cascade of apoptotic changes.

OCHRATOXIN A

Lipid peroxidation due to OTA consumption may be implicated in DNA damage. When a specially designed oxidation system was used, OTA was found to facilitate single-strand

cleavage of supercoiled plasma DNA through ROS production (Gillman *et al.,* 1999). In general, OTA is implicated in DNA-adduct formation (Grosse *et al.,* 1997). OTA treatment of rats resulted in a 5-fold increase in the expression of the protein haem oxygenase-1 generated during oxidative stress (Gautier *et al.,* 2001). Changes in redox status of the cell due to the pro-oxidant action of OTA could trigger cell apoptosis. For example, OTA at a dose of 20 mg/kg caused nuclear changes characteristic of apoptosis in hamster kidney (HaK) and HeLa cells (Seegers *et al.,* 1994). Furthermore, the results indicated that OTA might activate different cellular processes involved in the degradation of DNA in HaK and HeLa cells. Exposure of human proximal tubule-derived cells to 30 nmol/L or more OTA led to DNA fragmentation and chromatin condensation (Schwerdt *et al.,* 1999). It was concluded that exposure to low OTA concentrations can lead to direct or indirect caspase-3 activation and subsequently to apoptosis in cultured human proximal tubule cells and in other renal epithelial cell lines of different origins.

Administration of OTA twice a week for one or two weeks resulted in apoptosis in the liver of mice (Atroshi *et al.,* 2000). In particular, the presence of intracellular apoptosis bodies was detected two weeks after toxin treatment. Light microscopic examination demonstrated the presence of eosinophilic globules, often containing apoptotic bodies. They were found within the cytoplasm of intact hepatic cells. The number of apoptotic bodies was further enhanced at two weeks, resulting in an 8-fold increase in liver over the control values (Atroshi *et al.,* 2000). Wistar rats were treated with a low dose (5 mM and 1 mg/kg, respectively) or a high dose (12.5 mM and 10 mg/kg, respectively) of OTA for 24 or 72 hrs. Marked treatment-specific transcriptional changes were detected for genes involved in DNA damage response and apoptosis (Luhe *et al.,* 2003). Because of OTA consumption, there was a striking increase in the counts of eosinophils and of apoptotic phagocytes in weaner pigs in a dose-dependent manner (Muller *et al.,* 1999).

Nanomolar concentrations of OTA promote apoptosis in a cell-type specific fashion. For example, OTA, at noncytotoxic doses, was able to detach collagen- and fibronectin-adherent cells from immobilized substratum causing apoptosis as measured by caspase-3 activation (Scibelli *et al.,* 2003). Relatively low OTA concentrations (547.2, 752.5 and 930.3 ng OTA/g kidney tissue) have activated apoptotic processes and oxidative damage in kidney cells (Petrik *et al.,* 2003). In human monocyte/macrophage line THP-1 metabolic activity, cell proliferation, cell membrane integrity, cell differentiation, phagocytic behaviour, NO synthesis and cell surface markers were largely suppressed by OTA at concentrations between 10 and 1000 ng/ml (Muller *et al.,* 2003). OTA greatly induced apoptosis in human myc-transfected cell line (Horvath *et al.,* 2002).

Exposure to low OTA concentrations (5-30 nmol/L) can lead to time- and concentration-dependent direct or indirect caspase-3 activation and subsequently to apoptosis in cultured human proximal tubule cells and in other renal epithelial cell lines of different origins (Schwerdt *et al.,* 1999). Additional evidence was provided indicating that OTA interacts in a cell type-specific way with distinct members of the mitogen-activated protein kinase family providing induction of apoptosis via the c-jun amino terminal kinase pathway (Gekle *et al.,* 2000).

OTHER MYCOTOXINS

When the *in vitro* effect of deoxynivalenol (DON) on apoptosis in specific T- and B-cell subsets within thymus, spleen and Peyer's patch cultures was studied (Pestka *et al.,* 1994) it was shown that depending on lymphocyte subset, tissue source and glucocorticoid induction, DON could either

enhance or inhibit apoptosis. Combined treatment of mice with DON and lipopolysaccharide significantly increased the amount of apoptotic thymic and splenic tissue (Zhou *et al.,* 2000). It was suggested that inhibition of protein synthesis and induction of apoptosis are the main mechanisms of DON toxicity in intestinal cells (Maresca *et al.,* 2002).

It seems likely that DON can interact with other inducers of apoptosis to increase apoptotic action. As demonstrated by DNA fragmentation and flow cytometric analysis, apoptosis in thymus, Peyer's patches, and bone marrow was marked in mice 12 hrs after administering *Escherichia coli* LPS (0.1 mg/kg BW i.p.) concurrently with DON (12.5 mg/kg BW p.o.), whereas apoptosis in control mice or mice treated with either toxin alone was minimal (Islam *et al.,* 2002). Therefore, lipopolysaccharide can interact with DON in mice to induce the glucocorticoid-driven apoptotic loss of immature thymocytes and cytotoxic T-lymphocytes in thymus, mature-B-lymphocytes in Peyer's patch, and pro/pre-B-lymphocytes and mature B-lymphocytes in bone marrow in mice (Islam *et al.,* 2003).

Dead lymphocytes in the thymus, spleen and Peyer's patches showed ultrastructural characteristics of apoptosis. DON and aflatoxin G_1 (AFG_1) could induce and accelerate apoptosis in human peripheral blood lymphocytes. For example, a typical sub-diploid apoptosis peak was demonstrated in lymphocytes treated with DON and AFG_1 (Wang *et al.,* 1999). In particular, a significant dose response effect and time effect correlation was found between apoptosis rates and mycotoxin concentrations and the treated time.

In an experiment with male rats, AFB_1 was administered i.p. or intratracheally at 2-8 mg/kg BW and animals were killed after 26 hrs (Raj *et al.,* 2001). Dose-dependent induction of micronuclei in bone marrow and lung cells was observed. Furthermore, apoptotic bodies were found in lung cells of rats given AFB_1, which was correlated with micronuclei induction. Clearly, AFB_1 induced micronuclei and apoptosis in lung and bone marrow cells. This stimulation could be associated with the stimulation of the phosphatidylinositol (PI) cycle and activation of PI kinase by AFB_1 (Pasupathy *et al.,* 1999). Apoptotic rates in rat liver were significantly reduced when melatonin was co-administered with AFB_1 (El-Gibaly *et al.,* 2003). Rats were treated with AFB_1 (50 mg/kg BW) for 8 weeks. The levels of caspase-3 activity in the AFB_1 group were significantly higher than in controls and the apoptosis was associated with degenerative and necrotic changes in the hepatocytes (Meki *et al.,* 2001a). In the study, caspase-3 activity was positively correlated with MDA while negatively correlated with GSH, GSH-Px and GSH-reductase in rat livers treated with AFB_1. It is interesting that melatonin treatment of rats could enhance hepatic antioxidant/detoxification system, which consequently reduced the apoptotic rate and the necrobiotic changes in the liver.

It seems likely that apoptosis is the principal mechanism contributing to germ cell depletion and testicular atrophy following zearalenone (ZEA) exposure. In particular, Kim *et al.* (2003) demonstrated that a single dose of ZEA (5 mg/kg) induced testicular germ cell apoptosis in a time-dependent and stage-specific pattern. In another study, ZEA (10, 20 and 40 mM) induced concentration-dependent DNA fragmentation in three cell lines (Vero, Caco-2 and DOK) resulting in DNA laddering patterns on agarose gel electrophoresis. This observation was consistent with apoptosis, which was confirmed by observations of formation of apoptotic bodies. Furthermore, ZEA induced cell cycle arrest in the three cell lines characterised by an increase in the number of cells in the G2/M phase of the cell cycle (Abid-Essefi *et al.,* 2003). It is interesting that vitamin E (25 mM) added simultaneously with ZEA partially reduced DNA fragmentation and apoptotic body formation after 24 hrs incubation.

Mycotoxins and the immune system

All animals protect themselves from invasion by microorganisms, parasites, fungi, viruses and any foreign molecules. This protective capacity requires an effective immune system, which is an important determinant of animal health and well being. In that sense, the remarkable ability of the immune system to distinguish between self and non-self is a great achievement of evolution. Commercial animal production is based on balanced feed, providing nutrient requirements and optimised environmental conditions. However, it is very difficult to avoid various nutritional or environmental stresses, which are responsible for immunosuppression and increased susceptibility to various diseases and consequently decreased productive and reproductive performance of farm animals. In this respect mycotoxins are one of the most immunosupressive factors in animal diets.

There are two major types of immune function: natural and acquired immunity (Figure 2). Natural immunity, called the innate immune system, includes physical barriers (e.g. skin, mucus layer of the gastrointestinal tract), specific molecules such as agglutinins, precipitins, acute phase proteins and lysozyme, phagocytic destruction of pathogens by macrophages and neutrophils, and lysing activity by lymphocytes called natural killer (NK) cells.

Macrophages perform a range of functions, including phagocytosis of foreign particles, destruction of bacterial or tumor cells, secretion of prostaglandins and cytokines, and as a result regulating activity of lymphocytes and other macrophages (Qureshi, 1998). In fact, phagocytosis is the major mechanism by which microbes are removed from the body and is especially important for defence against extracellular microbes. As a result of stimulation (for example by microbes) monocytes differentiate into macrophages that are more powerful in mediating host defence (Klasing, 1998a).

Macrophage activation and phagocytosis of foreign particles are regularly accompanied by a **respiratory burst**, an increase in the production of reactive oxygen species (ROS), exerted by the enzyme complex NADPH oxidase. Therefore macrophages as well as

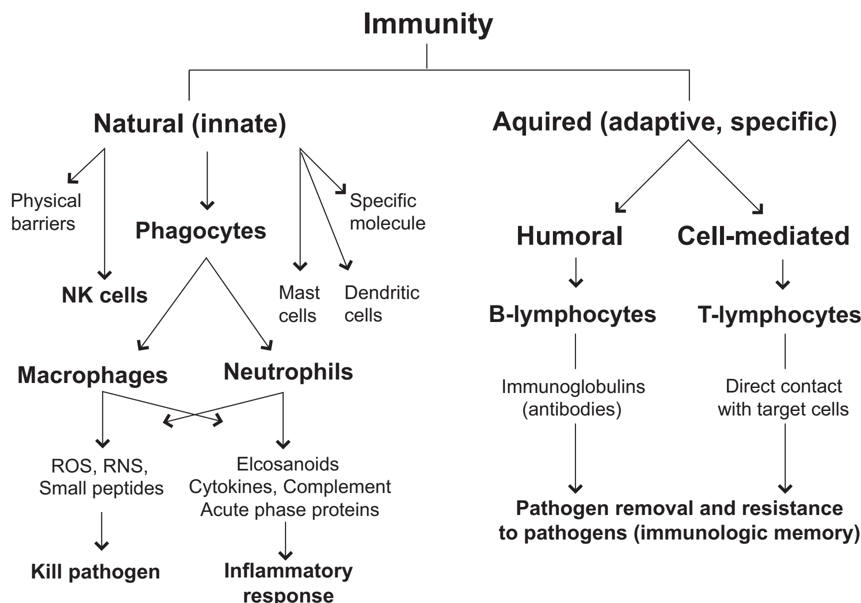

Figure 2. General overview of the immune system.

other phagocytic leukocytes (e.g., neutrophils, monocytes and eosinophils) can synthesize toxic oxygen metabolites such as superoxide anion (O_2^-), hydroxyl radical ($OH^\bullet$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitric oxide (NO) and peroxinitrite ($ONOO^-$) during the respiratory burst (Zhao *et al.,* 1998). For example, a bacterium coming into contact with the plasma membrane is enclosed in a plasma membrane vesicle containing NADPH oxidase, and exposed to an intensive flow of superoxide radical (Giller and Sigler, 1995). Superoxide radical can dismutate to H_2O_2, which penetrates the bacterium with production of hydroxyl radical, which is ultimately deadly to biological molecules. In general, the production of ROS and reactive nitrogen species (RNS) is a characteristic of both mammalian and avian macrophages (Qureshi *et al.,* 1998a).

In general ROS, RNS and eicosanoids (e.g. leukotrienes and prostaglandins) have recently received substantial attention as major metabolites produced by macrophages (Dietert and Golemboski, 1998). Because of this powerful weapon, macrophages bind, internalize, and degrade foreign antigens (e.g. bacteria) quite quickly. It takes only 15 minutes for chicken macrophages to kill more than 80% of the internalized *Salmonella* (Qureshi *et al.,* 1998a). Therefore natural immunity works rapidly, and gives rise to the acute inflammatory response. Macrophages contain various substances (including enzymes producing free radicals and small peptides with antibiotic activity) involved in microbial killing. They also have receptors for chemoattractive factors released from microbes. In addition to ROS, RNS and eicosanoids mentioned above, macrophages also synthesize and secrete a great number of cellular communication molecules such as cytokines, including the pro-inflammatory cytokines interleukin 1 (IL-1), interleukin 6 (IL-6), and TNF-α. They also produce cytokine inhibitors, hormones and neurotransmitters (Klasing, 1998a), which regulate specific immunity (initiating and directing the immune and inflammatory responses) and many other related physiological responses. Therefore macrophages are important amplifiers of the immune response, both by cytokine production and by serving to present parasite derived peptides to T-cells.

Acquired or specific immunity consists of humoral and cell-mediated immunity. There are two major types of lymphocytes, B-cells and T-cells. Humoral immunity is mediated by antibodies that are released by B-cells into the bloodstream. The bursa of Fabricius is the site of B-lymphocyte development and differentiation in birds. This immunity is based on the production of immunoglobulins. They are responsible for recognition and elimination of specific antigens: they bind and remove from the host invading organisms/ substances.

Cell-mediated immunity is based on specific antigen recognition by thymus-derived T-lymphocytes. Due to this immunity cells infected with a foreign agent, for example virus, are destroyed via direct contact between an activated T-cell and the target (infected) cell (Qureshi *et al.,* 1998). Cell-mediated immunity is responsible for delayed-type hypersensitivity (DTH) reactions, foreign graft rejections, resistance to many pathogenic microorganisms and tumor immunosurveillance (Wu and Maydani, 1998).

In birds, both T-cell and B-cell precursors originate in the bone marrow. Actual development of T-cells takes place in the thymus and B-cells develop in the bursa of Fabricius in birds and in bone marrow of mammals (Lahtala, 1998). Interactions between T- and B-cells, as well as antigen presenting cells, are responsible for the development of specific immunity. The defence mechanisms of specific immunity are induced or stimulated by exposure to foreign substances, are specific for distinct macromolecules and increase in magnitude with each successive exposure to a particular macromolecule (Miles and Calder, 1998). In

comparison to natural immunity, specific immunity takes longer to develop, but is highly specific for antigens and has memory. These two parts of the immune system work together through direct cell contact and through interactions involving such chemical mediators as cytokines and chemokines. Therefore, the animal immune system requires the co-operation of macrophages, bursa-derived B-lymphocytes and thymus-derived T-lymphocytes with various other types of cells.

The immune response involves cellular proliferation (T-lymphocytes), enhanced protein synthesis (including immunoglobulin synthesis by B-lymphocytes and acute phase protein synthesis in the liver) and inflammatory mediator production. Physiological changes resulting from stimulation of the immune system include fever, anorexia and loss of tissue components (Grimble, 1997).

When analysing data related to immunomodulation careful attention must be paid to methods used to assess immunological functions. For example, *in vivo* methods of immune function assessment are based on two main approaches: antibody response to vaccine or delayed-type hypersensitivity (DTH) reactions. In the first method, immunisation with appropriate antigens (viral or bacterial) can elicit serum antibodies. The haemagglutination (HA) assay measures serum antibody concentration (titre) against antigens, which are often sheep red blood cells (SRBC). This assay provides information about humoral immunity (B-cell responsiveness) and its association with cell-mediated immunity (T-cell co-operation). The second (DTH) method is used to assess cell-mediated immune function.

In vitro indices of immune function include (Wu and Meydani, 1998):

- *Lymphocyte proliferation assay*. This assay provides information about cell-mediated immune response and consists of measuring the number of cells in culture with or without addition of a stimulatory agent (mitogen). In this assay, isolated lymphocytes are incubated with mitogens, which activate division of either T-or B-lymphocytes. Various mitogens are used in such assays, but most often they include concanavalin A (con A, a T-cell mitogen), phytohemagglutin (PHA, a T-cell mitogen), lipopolysaccharide (LPS, a B-cell mitogen) and pokeweed mitogen (PWM, T- and B-cell mitogen) (Hayek *et al.,* 1996). Decreased proliferation may indicate impaired cell-mediated immunity.
- *Cytokine production*. T-cells produce a range of protein mediators called cytokines, which regulate cell activation, growth, differentiation, inflammation and immunity.
- *Cytotoxicity assay*. This assay assesses activity of cytotoxic T-lymphocytes (a group of T-cells that kill other cells by recognising their cell-surface antigens) and NK cells (a group of non-T and non-B lymphocytes that kill virus-infected and tumour cells).
- *Flow cytometric analysis*. This assay identifies cells with different surface markers. The results can be used for understanding the cellular basis of immune response.
- *Plaque-forming cell (PFC) test*. This shows the number of antibody-producing cells.

The impact of the avian immune system in modern production cannot be overestimated. The banning of feed grade antibiotics in Europe has made immunocompetence the major factor determining efficiency of poultry production. Molecular immunology is developing very quickly; and mechanisms and factors affecting avian immunocompetence have recently received substantial attention (McCorkle, 1998; Saif and Swayne, 1998) and nutritional modulation of resistance to infectious diseases in poultry (Klasing, 1998) is a frontline for future research. It must be emphasised that cellular integrity is very important for receiving and

responding to the messages needed to co-ordinate an immune response (Latshaw, 1991). Therefore, the antioxidant/pro-oxidant balance of the host is a critical consideration in optimal immune system function.

The clinical toxicologic syndromes caused by ingestion of mycotoxins have been characterized in domestic animals, poultry and laboratory animals and range from acute mortality to decreased performance and immunosuppression. In fact, consumption of certain mycotoxins, at levels that do not cause overt clinical mycotoxicosis, suppresses immune function and decrease resistance to infectious disease (Corrier, 1991). The effects of several mycotoxins on immune responses have been investigated (Tables 5-9); however, most data were obtained with laboratory animals. In some instances, farm animals and cells derived from livestock species have been employed to evaluate the immunotoxicity of mycotoxins (Sharma, 1993; Bondy and Pestka, 2000).

Immunosupressive potency of various mycotoxins differs substantially. Effects of DON, 3-acetyldeoxynivalenol, fusarenon-X, T-2 toxin, ZEA, α–zearalenol, ß-zearalenol and NIV on T- and B-cells were studied using a proliferation assay and antibody-dependent cellular cytotoxic NK cell activity on human peripheral blood mononuclear cells (Berek *et al.,* 2001). The mycotoxin concentrations used in the experiments were comparable to those found in normal human peripheral blood (0.2-1800 ng/ml). Among the mycotoxins tested, T-2 toxin, fusarenon-X, NIV and DON showed the highest immunosuppressing effects *in vitro*. In particular, mycotoxin-induced immuno-suppression was related to depressed T- or B-lymphocyte activity. Furthermore, they also inhibited NK cell activity (Berek *et al.,* 2001).

As can be seem from Table 5, immunosuppression caused by AFB_1 has been demonstrated in various livestock species (e.g., chickens, turkeys, pigs and lambs) and also in laboratory animals (mice and rats) and in various *in vitro* systems. Aflatoxin is an immunomodulating agent that acts primarily on cell-mediated immunity and phagocytic cell function. Therefore, AFB_1 mainly decreases lymphocyte activity and may affect macrophages assisting lymphocyte function. For example, in macrophages exposed to AFB_1, NO production stimulated by LPS significantly decreased (Moon *et al.,* 1998). Furthermore, AFB_1 decreased pro-inflammatory and increased anti-inflammatory cytokine mRNA expression in weanling piglets (Marin *et al.,* 2002). AFB_1 can be transferred from chicken to the egg and further to the developing embryo. Therefore, the progeny of hens consuming an AFB_1-contaminated diet may be increasingly susceptible to disease owing to suppression of humoral and cellular immunity. For example, anti-*Brucella abortus* antibody production was compromised and ROS production by macrophages from AFB_1 progeny decreased (Qureshi *et al.,* 1998). Long-term immune depression of macrophage-mediated functions can occur following embryonic exposure to AFB_1 (Neldon-Ortiz and Qureshi, 1992). Furthermore, depressed complement and interferon production could also result from AFB_1 exposure. Therefore, acquired immunity from vaccination programs may be substantially suppressed; and in such cases the signs of disease observed are those of the infectious process rather than those of the aflatoxin that predisposed the animal to infection. Mixtures of aflatoxin with other mycotoxins can result in greatly augmented biological responses in terms of weight gain depression, lethality, and immune reactivity (Pier, 1992).

The immunomodulatory effects of ochratoxins have also been considered for many years and a summary is shown in Table 6. OTA has been shown to affect mainly humoral immune function at the level of antibody synthesis in chickens, rats and mice. However, number and phagocytic activity of macrophages were also decresed in growing gilts receiving OTA for 35 days (Harvey *et al.,* 1992). IL production was also compromised. It has been demonstrated that exposure of purified

Table 5. Effects of aflatoxin B_1 on immunity.

Species	*Dose*	*Effects on immune system*	*Reference*
Broiler chicks	1 mg/kg, 7-49 days	Titres to NK ↓	Shivachandra *et al.,* 2003
Broiler chicks	2.5 mg/kg diet for 21 days	Peripheral T-lymphocyte counts ↓, Splenic plasma cell counts ↓	Celik *et al.,* 2000
Broiler chicks	2.5 mg/kg diet for 21 days	Percentage/mean phagocytic activity ↓; Immune response measured by HI test ↓	Ibrahim *et al.,* 2000
Broiler breeder hens	0.2; 1; 5 or 10 mg/kg	In progeny, anti-*Brucella abortus* titres ↓; Phagocytosis of SRBC and ROS production by macrophages ↓ in 5 mg AFB_1/kg	Qureshi *et al.,* 1998
Broiler chicks	5 mg/kg	Secondary antibodies against IBD↑	Okotie-Eboh *et al.,* 1997
Broiler chicks	400 μg/kg AFB_1 + AFB_2 for 5 weeks	Cell-mediated immunity measured by DTH skin test ↓; Humoral immunity unchanged; Acquired immunity to ND or fowl cholera vaccination unchanged	Giambrone *et al.,* 1985a
Broiler chickens	2.5 μg/g of feed for 42 days	Total complement activity ↓	Stewart *et al.,* 1985
Chickens	2.5 μg/g diet from hatching to 4 weeks of age	Cell-mediated immunity (graft-vs. host reaction) ↓; DTH reactions to tuberculin ↓; Humoral immunity (to rabbit RBC) unchanged; Serum IgG and IgA↓; IgM unchanged	Giambrone *et al.,* 1978
Chickens	0.3 mg/kg from 0 to 6 weeks	Cell mediated immune response (DTH reaction) suppressed at 30, 45 and 60 days of age	Kadian *et al.,* 1988
14-day-old turkeys and	200 μg/kg	Cell-mediated immunity (DTH skin test) ↓; Humoral immunity unchanged; Acquired immunity to ND or fowl cholera vaccination unchanged	Giambrone *et al.,* 1985b
4-month-old wild turkeys	100-400 μg/kg feed for 2 wk	Lymphoblast transformation ↓	Quist *et al.,* 2000
Chick embryo	1.09-17.4 μg/g embryo at day 18 of incubation	Mitotic index B-cells ↓; Mitotic index T-cells unchanged; Sister chromatid exchanges in B-lymphocytes ↑; Sister chromatid exchanges in T-lymphocytes ↑	Potchinsky and Bloom, 1993
6-day chick embryos	0.1, 0.5, and 1 μg/per embryo	Macrophages recruited in the peritoneal cavity after i.p. Sephadex elicitation ↓; Substrate adherence potential of peritoneal exudate cells ↓ at 1 μg AFB_1; Macrophage phagocytic potential ↓ at 0.5-1.0 μg AFB_1	Neldon-Ortiz and Qureshi, 1992

Table 5. Continued.

Species	*Dose*	*Effects on immune system*	*Reference*
6-day chick embryos	0.1 µg	Incidence of sister chromatid exchanges in blood cells ↑ (5-fold); Cell-mediated immunity (graft vs host and cutaneous basophil hypersensitivity reactions) ↓	Dietert *et al.*, 1985
Weanling piglets	140-280 µg/kg, 4 wks	AFB_1 decreased proinflammatory (IL-1ß, TNF-α) and increased anti-inflammatory (IL-10) cytokine mRNA expression	Marin *et al.*, 2002
Weaned pigs	140, or 280 µg/kg, for 3 wks	Skin thickness (PHA test) ↓ linearly with ↑ dietary AFB_1	van Heugten *et al.*, 1994
Pigs	300 or 500 mg/kg of feed	Humoral immune response to *Erysipelothrix rhusiopathiae*, measured by ELISA unchanged; Proliferative responses to mitogens unchanged; Complement titers↓; Serum IgG and M values↑	Panangala *et al.*, 1986
Female lambs	2 mg/kg for 37 d	Response to intradermal injection of PHA↓	Fernandez *et al.*, 2000
White-tailed deer	0.8 mg/kg for 8 wks	Lymphocyte proliferation and DTH reactions unchanged	Quist *et al.*, 1997
Weanling rats	60, 300 and 600 µg/kg BW	Cell mediated immunity, measured by DTH response assay, ↓ at the 300 and 600 µg dose levels	Raisuddin *et al.*, 1993
Weanling rats	350 and 700 µg/kgBW	Population and phagocytic capacity of macrophages ↓	Raisuddin *et al.*, 1990
Rats and mice	Aerosol inhalation or intratracheal instillation to AFB_1	Alveolar macrophage phagocytosis ↓ at 16.8 µg/kg with the effect persisting for ~2 weeks	Jakab *et al.*, 1994
Mice	0.03, 0.145 or 0.70 mg/kg orally every other day for 2 weeks	$[^3H]$thymidine uptake in lymphocyte cultures↓; Synthesis of DNA in mixed lymphocyte ↓; Primary antibody production by splenic cells from animals challenged with SRBC; DTH reaction to hemocyanin ↓	Reddy *et al.*, 1987
Murine peritoneal macrophages	10 or 50 mM	NO production stimulated by LPS ↓, mediated by the reduction of iNOS activity, mRNA, and protein	Moon *et al.*, 1998
Human monocytes	0.1-1 pg/ml	Phagocytosis and microbicidal activity ↓, Intrinsic antiviral activity or superoxide production unchanged	Cusumano *et al.*, 1996
Rat Kupffer cells *in vitro*	0.01 pg/ml	Phagocytosis ↓, intracellular killing of *Candida albicans* ↓, intrinsic anti-Herpes virus activity ↓	Cusumano *et al.*, 1995

Table 6. Effect of Ochratoxin A on immunity.

Species	*Dose*	*Effects on immune system*	*Reference*
Broiler chicks	2 mg/kg	Vaccination titres to NK ↓, Mitotic cells in the bursa ↓	Santin *et al.,* 2002
Chicks	5 mg/kg	Weight of lymphoid organs ↓, Humoral immune response ↓	Stoev *et al.,* 2002
Broiler chicks	130, 305 and 790 mg/kg	HI titers to ND vaccination ↓	Stoev *et al.,* 2000
Broiler chicks	Up to 4 mg/kg for 20 days from hatch	Ig-containing cells in lymphoid organs ↓; Total Ig levels ↓ at 2-4 ppm	Dwivedi and Burns, 1984
Broiler chickens	0.5-8 μg/g from day-old to 3 weeks of age	Total circulating lymphocytes ↓; Monocytes ↓ at 2.0 μg/g; Circulating heterophils unchanged	Chang *et al.,* 1979
Salmonella-challenged broiler chicks	3.0 mg/kg	PHA- and ConA-stimulated blastogenesis ↓; *S. typhimurium* alone had no effect on the variables measured	Elissalde *et al.,* 1994
Growing gilts	2.5 mg/kg of feed for 35 days	Cutaneous basophil hyper-sensitivity response to PHA ↓, DTH to tuberculin ↓, Stimulation index for lymphoblastogenesis ↓, Il-2 production when lymphocytes were stimulated with ConA ↓, Number and phagocytic activity of macrophages ↓	Harvey *et al.,* 1992
Rats	Gavage with 0, 0.07, 0.34 or 1.68 mg OTA/kg BW for 4 wks	Thickening of the basement membrane and reduction in splenic T-cell fraction. IgG ↓ at 0.34-1.68 mg/kg OTA	Dortant *et al.,* 2001
Rat	Dam's exposure to a single dose of OTA (0, 10, 50 or 250 mg/kg BW) on day 11 of lactation	The proliferative response of splenocytes to ConA ↑ in pups from dams given 10 or 50 μg OTA/kg BW; Proliferation of thymocytes in response to ConA ↑ in pups from dams exposed to 50 μg OTA/kg BW	Thuvander *et al.,* 1996b
Mice	Dams, OTA, 200 μg/kg before and during gestation	In offspring on days 14 and 28 postpartum percentages of splenic CD4+ and CD8+ cells ↓	Thuvander *et al.,* 1996
Mice	Dam's exposure to OTA (500 μg/kg BW) on day 16 of gestation	Proliferation of splenic and thymic lymphocytes in response to mitogens in pups at 15 days of age ↓; percentages of mature CD4+ cells ↓ and percentages of immature, double-positive (CD4/CD8+) cells in the exposed pups ↑	Thuvander *et al.,* 1996a
Mice	Dam's exposure to OTA (500 μg/kg BW) on day 10 postpartum	Proliferative responsiveness of lymphocytes in the offspring when stimulated with B- or T-cell mitogens 3 days after exposure ↑	Thuvander *et al.,* 1996a

Table 6. Continued.

Species	*Dose*	*Effects on immune system*	*Reference*
Mice	250 or 2600 mg/kg diet, 28 days	After 28 days, AB production and plaque-forming cells ↓; Decrease in thymocyte cell counts ↓ in the 250-μg/kg group; After 90 days, proportion of mature CD4+ or CD8+ cells ↓	Thuvander *et al.,* 1995
Mice	0.005 μg/kg BW	Immune response to SRBC ↓	Haubeck *et al.,* 1981

human lymphocyte populations and subpopulations to the toxin will abrogate the cell's ability to respond to activating stimuli *in vitro* (Lea *et al.,* 1989). Thus, both IL-2 production and IL-2 receptor expression of activated T-lymphocytes were severely impaired. The results strongly suggest that the toxin caused immunosuppression through interference with essential processes in cell metabolism (Lea *et al.,* 1989). In particular, OTA appears to suppress NK cell activity by inhibiting production of basal interferon. (Luster *et al.,* 1987).

It is important to note that subchronic oral exposure to OTA affects certain immune functions in mice at exposure levels that may be found in contaminated food products (Thuvander *et al.,* 1995). In the mouse model OTA had a non-selective suppressive effect on various immune reactions. They include weight depression, lymphopenia, neutrophilia and eosinophilia. Furthermore, antibody-producing cells, antibody titres in blood serum and phagocytosis of *E. coli* by blood phagocytes become suppressed. Immunized animals also showed a lower survival rate after experimental infection with *Pasteurella multocida* as well as an increase in oxygen radicals in blood cells (Muller *et al.,* 1995). Similarly, in *Salmonella*-challenged broiler chicks PHA- and ConA-stimulated blastogenesis was depressed as a result of OTA consumption, while *Salmonella* alone had no effect on these parameters (Elissalde *et al.,* 1994).

In weaner pigs subtoxic amounts of OTA produced immunomodulation in a dose-dependent mode (Muller *et al.,* 1999). It included increased counts of total leukocytes and neutrophils in the blood and reduced lymphocyte levels. There was also a substantial increase in the counts of eosinophils and of apoptotic phagocytes. Reduced phagocytosis and reduced expression of a swine-specific surface marker on lymphocytes were also observed.

It is interesting that the time of exposure significantly influences the immunotoxic effects of OTA on the developing immune system in rodents (Thuvander *et al.,* 1996a). Recent studies have examined immune function in the offspring of rats and mice exposed to OTA pre- and perinatally (Bondy and Pestka, 2000). It has been shown that the decrease in proliferation and antibody production in young animals resulted from prenatal modulation of the immune system. In particular, prenatal exposure to relatively low doses of OTA may induce immuno-suppression in the progeny. In contrast, short-term exposure of suckling pups to OTA via the milk stimulated the proliferative responses of lymphocytes to polyclonal activation.

Fumonisin toxicity has been characterized relatively recently in comparison to aflatoxin and ochratoxin (Table 7), and fumonisin-induced immunotoxicity is an area of active research (Bondy and Pestka, 2000). In fact, FB_1 has diverse effects on the immune system, causing both stimulation and suppression of responses to foreign antigens, and apparently inducing an antigenic response to FB_1. For example, in chickens FB_1 caused a decrease in total immunoglobulins, in IgG and macrophage phagocytic activity (Qureshi

Table 7. Effects of Fumonisins on immunity.

Species	*Dose*	*Effects on immune system*	*Reference*
White Leghorn chicks	6.1, 10.5, and 42.7 mg/kg from day 7 to 42	Splenic, thymic, and liver weights ↓; Bursa of Fabricius unchanged; Total Ig and IgG; Macrophage phagocytic activity ↓; NK cell activity unchanged	Qureshi *et al.,* 1995
Turkey poults	75 mg/kg feed and AB_1, 200 μg/kg feed, from day 1 to 21	Primary immune response to SRBC ↑ (combination of FB_1 and AFB_1); PHA response unchanged	Weibking *et al.,* 1994
Weaned pigs	1-100 mg/animal/d	Titres unchanged; Lymphocyte stimulation by PHA, ConA, LPS unchanged	Tornyos *et al.,* 2003
Rat	Injected at 7.5 or 10 mg/kg BW/day for 4 days	Thymus weight ↓, Serum IgM ↑; Circulating phagocytic cell numbers ↑	Bondy *et al.,* 1995
Mice	50 (LD), FM1 + FM_2, 6 weeks	After challenge with *T. cruzi,* NO production by MG↑ after 14 days	Dresden *et al.,* 2002
Mice	Five daily s.c. injections of 2.25 mg/kg/d FB_1	Relative spleen and thymus weights in F ↓, No effect on organ weights in M; Splenic cellularity and the basal rate of lymphocyte proliferation in F ↓; PHA-induced T-lymphocyte and LPS-induced B-lymphocyte proliferation in F ↓; Expression of IL-2 mRNA in splenocytes in F ↓	Johnson and Sharma, 2001
Mice	I.p. with SRBC, at 5-100 μg	Number of plaque-forming cells produced against SRBC ↓	Martinova *et al.,* 1995
Mice	I.p daily, at 1 to 50 μg	4 to 12-fold increase in the number of of plaque-forming cells after SRBC injection	Martinova *et al.,* 1995
Macrophage cell line	1-10 μM	Membrane fluidity ↑; Membrane peroxidative damage ↑	Ferrante *et al.,* 2002
Murine macrophage cell line	1-100 μM	LPS-induced production of NO and PGE_2↑ at 0.1-10 μM	Meli *et al.,* 2000
Murine macrophage cell	1, 10, and 100 μM	LPS-induced NO production ↑	Rotter and Oh, 1996
Rat splenic macrophages and lymphocytes	1-100 μg/ml	NO production ↑; ConA-induced proliferation of splenic cells in the presence of NO synthase inhibitor ↑	Dombrink-Kurtzman *et al.,* 2000

et al., 1995). However, in turkey poults a combination of FB_1 and AFB_1 was responsible for increased primary immune response to SRBC (Weibking *et al.,* 1994). In weaned pigs, FB_1 (up to 100 mg/animal/day) did not affect immunity (Tornyos *et al.,* 2003). Effects of FB_1 on immunocompetence of laboratory animals also vary substantially (Table 7).

Immunomodulatory properties of FB_1 probably depend on its effect on lipid metabolism, antioxidant/pro-oxidant balance and interactions with other factors. For example, FB_1 decreased CD3 receptor expression on the surface of thymus cells *in vitro* and *in vivo*, which is consistent with the sharp decrease in the ceramide content in this organ (Martynova *et al.*, 1995).

Similar to FB_1, the trichothecenes can both suppress and stimulate immune function (Table 8). Most of the research on T-2 toxin effects on immunity was perfomed with laboratory animals and there are only a few publications addressing immunomodulating properties of T-2 toxin in farm animals. Futhermore, the molecular basis of immune modulation by mycotoxins remains mostly unknown and continues to be an active area of research (Bondy and Pestka, 2000). It appears that trichothecenes are potent immunosuppressive agents that directly affect immune cells and modify immune responses because of tissue damage elsewhere. For example, sheep and calves treated with *Fusarium* T-2 toxin develop leukopenia and decreased function of peripheral lymphocytes (Sharma, 1993). In fact, exposure of experimental animals and humans to T-2 toxin has been shown to have a variety of immunosuppressive effects, including altered parameters of humoral immunity. It is well established that T-2 toxin is cytotoxic to lymphocytic cells *in vitro*, however, limited information is presently available regarding the contribution of such a mechanism to immunosuppression *in vivo*, or to potential immune cell targets. It seems likely that lymphocyte progenitors, in contrast to thymocytes, represent highly sensitive targets of T-2 toxin exposure, responsible for thymic atrophy (Holladay *et al.*, 1993). For example, subchronic T-2 toxin treatment of timed-pregnant B6C3F1 mice resulted in significant and selective depletion of fetal liver cells expressing low levels of surface CD44 and CD45 antigens, suggestive of possible lymphoid progenitor cell sensitivity to this agent (Holladay *et al.*, 1995). The authors showed that the precursors of B-cells might represent highly sensitive targets of T-2 toxin exposure. Impaired resistance to pathogenic microorganisms occurs after exposure to the trichothecenes T-2 toxin and DON. This may predispose food animals to infectious disease and could result in decreased productivity as well as increased animal-to-human transmission of pathogens such as *Salmonella* and *Listeria* (Pestka and Bondy, 1990). For example, mice challenged with *S. typhimurium* and then treated with T-2 toxin every other day for 10 days had markedly larger and more bacteria-related lesions in the spleen, kidney, and liver than animals challenged with *S. typhimurium* alone (Tai and Pestka, 1990). In great contrast, in mice exposed to T-2 toxin at 3 mg/kg BW by gavage, virulence of both *E. coli* and *S. auerus* decreased. Apparently there are species-specific features in sensitivity to trichothecenes. For example, recently it has been shown that T-2 toxin (up to 1 ppm) in the poult diet did not affect antibody production stimulated by various antigens (Sklan *et al.*, 2003).

Immunomodulating properties of DON have been studied mainly with rodents (Table 9). The evidence is quickly accumulating to show that DON can be immunosuppressive or immunostimulatory, depending upon the dose and duration of exposure. While immunosuppression is probably related to the inhibition of translation, immunostimulation can be a result of interference with various cellular regulatory mechanisms (Rotter *et al.*, 1996). For example, *in vivo* DON suppresses normal immune response to pathogens and simultaneously induces autoimmune-like effects. Characteristic effects of DON also include superinduction of cytokine production by T-helper cells (*in vitro*) and activation of macrophages and T-cells to produce proinflammatory cytokines. Although these effects have been largely characterised in the mouse, several investigations with DON suggest that immunotoxic effects are

Table 8. Effects of T-2 toxin on immunity.

Species	*Dose*	*Effects on immune system*	*Reference*
Young poults	Up to 1 mg/kg for 32 days	Antibody titres unchanged	Sklan *et al.,* 2003
7-week-old pigs	0.5, 1.0, 2.0 or 3.0 mg/kg diet for 3 weeks	Leukocyte count ↓; Proportion of T-lymphocytes ↓; Antibody formation ↓; Blastogenic transformation of lymphocytes ↓	Rafai *et al.,* 1995
9-10-week-old pigs	Aerosol, 390 μg/L	Lymphocyte count ↓, Neutrophil count ↑; ConA, PHA, and PWM blastogenic responses and H1 titers to SRBC↓	Pang *et al.,* 1988
9-10-week-old pigs	15 mg/kg	Blastogenic responses to ConA, PHA and PWM ↓ at early (3 to 5 days) and late (20 to 28 days) postdosing intervals; HA titer to SRBC unchanged	Pang *et al.,* 1987
Calves	0.6 mg/kg/day	Lymphocyte responses to mitogens ↓; Chemotaxic migration of neutrophils ↓	Buening *et al.,* 1982
Calves	0.6 mg/kg per day for 43 days	IgM and IgA ↓; IgG→	Mann *et al.*, 1982
Monkeys	Gastric intubation at 100 μg/kg BW/ day for 4-5 wk	Leucocyte counts↓ at the end of wk 4; Bactericidal activity of neutrophils ↓, T-cell number ↓; Lymphocyte transformation ↓, B-cell number ↓; IgG and IgM levels ↓	Jagadeesan *et al.,* 1982
Rats	2 mg/kg	In progeny, the cortex was atrophic while the medulla was proliferative. In the spleen, both B- and T-cells were impaired and responsiveness to PHA and LPS decreased	Lafarge-Frayssinet *et al.,* 1990
Mice	3 mg/kg BW by gavage	Virulence of both *E. coli* and *S. aureus* ↓	Cooray and Jonsson, 1990
Mice challenged with *S. typhimurium*	1 mg/kg every other day for 10 d	Markedly larger and more bacteria-related lesions in the spleen, kidney, and liver than animals challenged with *S. typhimurium* alone	Tai and Pestka, 1990
Mice	20 ng	Immune response to sheep RBC ↓	Holt and DeLoach, 1988
Mice	1.5 or 3.0 ppm in the diet for 16 months	T-lymphocyte-dependent humoral immunity unaffected	Schiefer *et al.*, 1987
Mice exposed to *Listeria*	4.0 mg/ kg of BW	Growth of *Listeria* ↑; Mortality ↑	Corrier and Ziprin, 1986

Table 8. Continued.

Species	*Dose*	*Effects on immune system*	*Reference*
Mice	20 ppm for 1- 4 weeks	Spleen proliferative responses to ConA ↓;	Friend *et al.*, 1983
Human lymphocytes	1 -5 ng/ml	50% inhibition in cell proliferation	Meky *et al.*, 2001

Table 9. Effects of DON on immune system.

Species	*Dose*	*Effects on immune system*	*Reference*
Growing pigs	0.6, 1.8 and 4.7 mg/kg	Secondary antibody response to tetanus toxoid ↓ dose-dependently	Overnes *et al.*, 1997
Young pigs	0.75- 3.00 mg/kg diet for 28 days	α-globulin levels, and antibody titers to SRBC↓	Rotter *et al.*, 1994
Mice	5 or 25 mg/kg BW	mRNAs for IL-1ß, IL-6, and TNF-α; the T-helper 1 cytokines IFN-γ and IL-2; and the T helper 2 cytokines IL-4 and IL-10 ↑	Zhou *et al.*, 1997
Mice	25 mg/kg for 8 or 24 ws	Serum IgA and microhematuria index ↑ after 4 to 8 wks and continued to increase with further exposure	Dong and Pestka, 1993
Mice	25 ppm for 4 and 8 wks	Total IgA ↑; Total IgG and IgM ↓	Rasooly ands Pestka, 1992
Mice	10-100 mg/kg in the diet	Serum levels of anti-SRBC antibodies ↓; Stimulation of B- and T-cells by mitogens ↓	Robbana-Barnat *et al.*, 1988
Weanling mice	0.5- 25.0 mg/kg over 8 wks	Total circulating white blood cells ↓;PMN neutrophils ↑; Serum IgM ↓; IgA ↑	Forsell *et al.*, 1986
Weanling mice	0.75, 2.5 or 7.5 mg/kg BW by gavage	Serum IgM to SRBC ↓; Plaque-forming cell numbers ↓	Tryphonas *et al.*, 1984
Murine peritoneal macrophages	0.1 ng/ml-1 μg/ml	Phagocytosis ↓; Microbiocidal activity ↓; Superoxide anion production ↓ at 1 ng/ml; Phagosome-lysozome fusion ↓ at 100 ng/nl	Ayral *et al.*, 1992
Lymphocytes *in vitro*	0.005-100 ng/ml	PHA-induced lymphocyte proliferation ↑ at 0.005- 0.5 ng DON/ml; at 50-100 ng/ml lymphocyte proliferation ↓	Miller and Atkinson, 1986
Human lymphocytes	216 ng/ml	50% inhibition in cell proliferation	Meky *et al.*, 2001
Murine macrophage cell line	25-100 ng/ml	Production of H_2O_2 after LPS stimulation ↑, but ↓ at 250 ng/m; NO production ↓ at 25-250 ng/ml	Ji *et al.*, 1998

also likely in domestic animals (Rotter *et al.,* 1996). Therefore, DON can selectively and concurrently upregulate or downregulate critical functions associated with activated macrophages. For example, a single DON exposure rapidly induces gene expression *in vivo* for a wide range of cytokines with apparently complete recovery occurring after 24 hrs. Elevated cytokine expression may play an important role in the patho-physiological effects of DON and other trichothecenes (Zhou *et al.,* 1997). Males appeared more susceptible than female mice to DON-induced IgA dysregulation and IgA nephropathy in terms of latency, threshold dose, and severity (Greene *et al.,* 1994). It is interesting that experimental dysregulation of IgA production and IgA nephropathy persisted up to four months after a discrete period of dietary DON exposure (Dong and Pestka, 1993).

Mycotoxin-induced immunosuppression is related to both natural and adaptive immunity:

- depressed T- or B-lymphocyte activity
- depressed NK cell activity
- suppressed immunoglobulin and antibody production
- reduced complement or interferon activity
- impaired macrophage functions

The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the vulnerability of the continually proliferating and differentiating cells that participate in immunomediated activities and regulate the complex communication network between cellular and humoral components (Corrier, 1991). In fact, high levels of polyunsaturated fatty acids in the immune cells and presence of sensitive receptors on cell surfaces make them an important target for free radical attack (Surai, 2002). As mentioned above, oxidative stress caused by mycotoxin consumption could be responsible for breaking effective communication between immune cells, which ultimately would misregulate the immune system and result in immuno-suppression. Although the molecular basis for many of the specific immunosuppressive effects of mycotoxins is presently unclear, inhibition of DNA, RNA and protein synthesis via a variety of different mechanisms appears to be directly or indirectly responsible for the immunosuppressive action of many mycotoxins (Corrier, 1991). Furthermore, detrimental effects on antioxidant defences, stimulation of lipid peroxidation and apoptosis and damage to immune cell receptors appear to be involved in the immunomodulation properties of mycotoxins. Consequences of oxidative stress for immunocompetence are shown in Figure 3.

Protective effect of antioxidants against mycotoxicoses

The wide range of mycotoxins that can contaminate animal feed and their differing chemical compositions makes protection against mycotoxin-related toxicity a difficult task. There are various approaches to control or combat mycotoxin problems. The simplest strategy is based on the prevention of mycotoxin formation in feeds by special management programmes including storage at low moisture levels and prevention of grain damage during processing (Dawson, 2001). However, modern agronomic technology cannot eliminate pre-harvest infection of susceptible crops by fungi (Wood, 1992). Therefore, this strategy can be only partially effective; and in countries with warm and humid conditions, this strategy can be quite costly.

Other strategies based on microbial or thermal inactivation of toxins, physical separation of contaminated feedstuffs, irradiation, ammoniation and ozone degradation have not been developed to an industrial level because they are either time-consuming or comparatively expensive (Dawson, 2001a). In recent years, nutritional manipulation has been actively used to

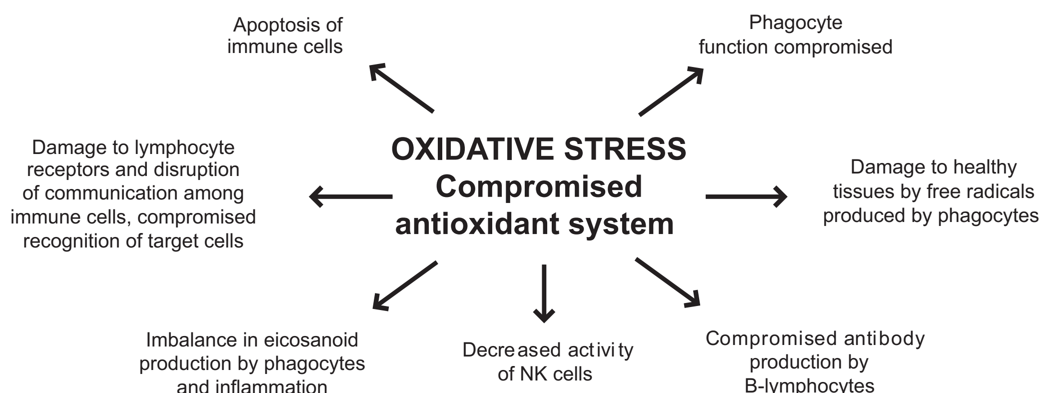

Figure 3. Oxidative stress and the immune system (Adapted from Surai, 2002).

improve animal defence against mycotoxins or to decrease the detrimental consequences of mycotoxin consumption.

Since lipid peroxidation plays an important role in mycotoxin toxicity, a protective effect of antioxidants is expected (Galvano *et al.*, 2001). Indeed, in several experiments with various animal species, protective effects of antioxidants against the toxic effects of mycotoxins were observed. For example, vitamin E supplementation ameliorated the pro-oxidative effects of OTA in the chicken (Hoehler and Maraquardt, 1996) and mouse (Grosse *et al.*, 1997). Trolox C, a water-soluble form of vitamin E, prevented the genotoxic effects of OTA in *E. coli* (Malavielle *et al.*, 1994). Protective effects of vitamin E were also obvious in aflatoxicosis (Shen *et al.*, 1994; Choi *et al.*, 1995; Souza *et al.*, 1999; Verma and Nair, 2001), DON or T-2 toxicoses (Segal *et al.*, 1983; Tsuchida *et al.*, 1984; Rizzo *et al.*, 1994) in rats; T-2 toxicosis in mice *in vivo* (Atroshi *et al.*, 1997) or in an *in vitro* system using cell lines (Shokri *et al.*, 2000). Vitamin E was also effective in protection against fumonisin B_1 (Abel and Gelderblom, 1998; Atroshi *et al.*, 1999) or ZEA (Grosse *et al.*, 1997; Ghedira-Chekir *et al.*, 1998; 1999). It also decreased the cytotoxic effect of T-2 toxin in cell culture (Shokri *et al.*, 2000). Pre-incubation of the glioma cells with vitamin E (25 mM) for 24 hrs before FB_1 (18 mM) significantly reduced DNA fragmentation and apoptotic bodies induced by FB_1 (Mobio *et al.*, 2000).

Other antioxidant compounds also have protective effects against various mycotoxins. Burguera *et al.* (1983) indicated that selenium (Se) has a protective effect against AFB_1 toxicity in turkey poults. To investigate the biochemical mechanism of the protective effect of dietary Se during aflatoxin exposure, hepatic metabolism of AFB_1 in turkey poults was examined at various dietary Se concentrations (0.2, 2.0 or 4.0 ppm). The experimental results provided clear evidence of Se-induced enhancement of aflatoxin detoxification (Gregory and Edds, 1984). It was suggested that the protective action of Se was not mediated by an increase in glutathione availability for aflatoxin conjugation or by effects on the activities of these enzymes as measured *in vitro*. Therefore dietary supplements such as Se are considered effective in the reduction of aflatoxicosis in poultry (Dalvi, 1986). Recently the protective antioxidant effect of organic Se in combination with vitamin E has been shown in chicks exposed to cold stress and aflatoxin-contaminated feed (Stanley, 1998).

Similar protective effects of Se against aflatoxicosis have been shown with mammalian species. Pigs fed a diet containing 2.5 mg Se/kg were protected from the toxic effects of AFB_1 (Davila *et al.*, 1983). The effects were measured by alteration of clinical responses and hematologic (prothrombin times), electrophoretic and clinical chemistry

values. Effects of a single intramuscular injection of Se-vitamin E (5 mg of Se + 68 IU of α–tocopherol/60 kg of BW) as a pre-treatment 14 days before an oral dose of AFB_1 (1.0 mg/kg) were studied in 24 dairy calves. Although aflatoxin exposure caused a significant decrease in body weight and feed intake, Se was found to interact significantly with AFB_1 for feed intake, causing an improvement in this parameter (Brucato *et al.,* 1986).

Milks *et al.* (1985) showed that Se is able to protect against the hepatocarcinogenic effects of AFB_1 in the rat. Moreover, inhibitory activity of Se on mutagenesis induced by AFB_1 in the presence of a rat liver microsomal activation system has been shown in *S. typhimurium* tests (Francis *et al.,* 1988). The results of another experiment showed that Se could effectively protect cells from AFB_1 cytotoxicity in cultured cells but had no effect on AFB_1-DNA adduct formation or mutagenesis (Shi *et al.,* 1995). In a different study, the same authors reported that Se could effectively inhibit AFB_1-induced DNA damage (Shi *et al.,* 1994).

It has been revealed that Se can inhibit the formation of hyperplastic foci and enzyme-altered foci as well as hepatocarcinogenesis induced by AFB_1, but Se can neither prevent the enlargement nor accelerate the regression of the foci already developed after administration of carcinogens (Wang, 1990). Therefore Lei *et al.* (1990) concluded that Se had an inhibitory effect on the initiation and promotion stages of AFB_1-induced preneoplastic foci and nodules. Selenium also prevented progression of these nodules to hepatocellular carcinoma even after cessation of AFB_1 administration. Additional experiments were conducted to verify the effect of Se on the mutagenic activity of AFB_1. After 14 days of Se administration to Chinese hamsters, the incidence of chromosomal aberrations in bone marrow cells due to a single administration of AFB_1 (5 mg /kg BW) was significantly reduced (Petr *et al.,* 1990). A significant decrease in the frequency of aberrant cells, breaks and gaps was observed throughout the investigation.

A protective effect of Se is not restricted to aflatoxins, but is obvious with T-2 toxin as well. When male Wistar rats were fed diets supplemented with Se (0.5 and 2.5 mg/kg) for six weeks, signs of intoxication from T-2 toxin were less distinct and mortality caused by T-2 toxin was 2-fold (Kravchenko *et al.,* 1990) or 3-5-fold (Tutelyan *et al.,* 1990) lower compared to the unsupplemented group. The acute lethal toxicity of T-2 toxin was reduced by administration of sodium selenite (Yazdanpanah *et al.,* 1997).

In summary, Se is shown to have protective effects against T-2 toxicity (Rizzo *et al.,* 1994; Shokri *et al.,* 2000; Kravchenko *et al.,* 1990; Tutelyan *et al.,* 1990; Yazdanpanah *et al.,* 1997), to decrease damaging effects of AFB_1 (Shen *et al.,* 1994; Choi *et al.,* 1995; Shi *et al.,* 1994; Shi *et al.,* 1995) and to prevent DON toxicosis (Rizzo *et al.,* 1994; Atroshi *et al.,* 1997). A synthetic seleno-organic compound (ebselen) showed a potent protective effect against AFB_1-induced cytotoxicity (Yang *et al.,* 2000). Indeed, as can be seen from data presented in Tables 1-4, protective effects against lipid peroxidation caused by mycotoxins were attributed to various antioxidant compounds including vitamins A and E, ascorbic acid, CoQ10, selenium, antioxidant enzymes as well as synthetic antioxidants and various plant extracts (Surai, 2002).

Conclusions

Recent results show that in many cases membrane-active properties of various mycotoxins determine their toxicity. Indeed, incorporation of mycotoxins into membrane structures causes various detrimental changes. These changes are associated with alteration of fatty acid composition of the membrane structures and with peroxidation of long chain PUFAs inside membranes. This ultimately damages membrane receptors, causing

alterations in second messenger systems; then to inactivation of a range of membrane-binding enzymes responsible for regulation of important pathways. Finally, this causes alterations in membrane permeability, flexibility and other important characteristics determining membrane function. Consequently, functional alterations in many biochemical pathways and changes in physiological functions including growth, development, reproduction etc. occur. An importance of lipid peroxidation in all these processes is confirmed by the protective effects of natural antioxidants against mycotoxin toxicity. However, molecular mechanisms of mycotoxin-antioxidant interactions *in vivo* await investigation.

Considering data reviewed, we can suggest a hypothetical scheme of mycotoxin-antioxidant interactions.

- Mycotoxins in the feed (at least OTA, T-2 toxin and AFB_1) cause malabsorption in the intestine, which in turn decreases concentrations of vitamin E, C, and carotenoids in tissues. Mycotoxins promote free radical formation (O_2^- and OH•) in the intestine, which cause antioxidant depletion, oxidative stress, enterocyte apoptosis and contribute to the development of malabsorption and decreased antioxidant absorption and accumulation.
- Mycotoxins and their active metabolites are absorbed from the intestine and accumulate in target tissues.
- Mycotoxins in tissues can generate free radicals, decreasing further antioxidant protection, causing lipid peroxidation and damage to other biological molecules including lipids, proteins and DNA. This could lead to antioxidant/pro-oxidant imbalance causing oxidative stress, which further leads to apoptosis and other cytotoxic effects of mycotoxins.
- Immunosupressive action of mycotoxins could be associated with downregulation of communications among various cells due to damage to receptors as well as downregulation of communicating molecule (cytokines, eicosanoids, etc.) production by macrophages.
- Increased antioxidant supplementation protects against toxic actions of mycotoxins by interfering with one or several steps described above, including gastrointestinal tract, plasma and tissue membranes.
- A combination of natural antioxidants with mycotoxin adsorbents could be a next step in combating mycotoxicoses in poultry production.

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